Supplemental information

Supplemental experimental procedures

Supplemental figures 1-5

Supplemental figure legends

Supplemental tables S1 and S2

Supplemental experimental procedures

Chromatin immunoprecipitation

Chromatin immunoprecipitation was essentially performed as previously described (Labbadia and Morimoto, 2015a). Approximately 10,000 bleach synchronized L1 larvae were grown on 10 cm RNAi plates seeded with RNAi bacteria (approx. 500 worms per plate). Animals were subjected to control or heat shock (33°C, 30 minutes) conditions at day 2 of adulthood by submerging parafilmed plates in a large water bath. Day 2 adults were then washed off large plates in M9 and allowed to gravity sediment for 1 -2 minutes. Eggs and L1 larvae suspended in the "supernatant" were removed and the process was repeated 3 times to ensure the removal of all progeny (confirmed by examining 20 µl aliquots of packed adult worms on a glass slide under a light microscope). Chromatin cross-linking was performed by gently agitating worms in 1% formaldehyde in PBS on a nutator at room temperature for 30 min with intermittent douncing of worms to promote disruption of the cuticle. Cross-linking was quenched by incubation with 125 mM glycine for 10 minutes at room temperature. Worms were then pelleted and washed 3 times with ice-cold FA buffer (50 mM HEPES/KOH pH7.5, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxyholate, 150 mM NaCl with Roche Complete protease inhibitors). Worm pellets were then re-suspended in 500 µl of FA buffer and dounce homogenized on ice for 30 - 60 minutes to completely break-up worms. Animals were further broken up by sonication on high power (30s on, 60s off) using a Bioruptor sonicator (Diagenode). Complete destruction of worms was confirmed by examination of 2 µl of worm chromatin samples under a light microscope and chromatin preps were then centrifuged at 17,000g for 15 min at 4°C. Chromatin was sheared to 200 - 500 base pair fragments with an additional 2 rounds of sonication on high power (30s on, 60s off) and shearing efficiency was examined by electrophoresis of 5 µl of reverse cross-linked chromatin on a 1% agarose gel. Pulldowns were set up using 2 mg of protein, 20 µl of protein-G dynabeads (Invitrogen), and either 5 µl of anti-full length GFP antibody (Clontech, living colors), or 2 µg of anti-AMA-1 antibody (Novus Biological) in FA buffer (1 ml total volume). Pulldowns were then incubated at 4°C overnight on a rotator. Following incubation, protein-G dynabeads were washed at room temperature 2 times for 5 minutes in FA buffer, then once for 10 minutes in FA buffer with 500mM NaCl, once for 5 minutes in FA buffer with 1M NaCl, once in for 10 minutes in TEL buffer (0.25M LiCl, 1% NP-40, 1% sodium deoxyholate, 1mM EDTA, 10mM Tris-HCl, pH 8.0), and twice for 5 minutes in TE pH 8.0. DNA was eluted twice by incubating beads with 50 µl elution buffer (TE containing 1% SDS and 250mM NaCl) at 65°C for 30 minutes each time. 10% of input

DNA was also diluted in 100 µl elution buffer and processed in parallel with ChIP samples. Eluted DNA was treated with RNase A for 30 minutes at 37°C and then with 0.1 mg/ml Proteinase K for 1 hour at 50°C. Crosslinking was then reversed at 65°C overnight and DNA samples were purified using Qiagen PCR purification columns. Levels of ChIP DNA relative to inputs were then determined by RT-qPCR using the relative standard curve method of quantification. Primers used can be found in Table S2.

Western blotting

Briefly, 50 – 100 day 2 adult animals were picked directly into 20 µl of Laemmli loading buffer and incubated at 95°C for 5 minutes. 5 µl of sample was then separated by SDS-PAGE and transferred to a nitrocellulose membrane. Blots were then blocked for 1 hour at room temperature with 5% non-fat milk in PBS (w/v) and washed 3 times in PBS 0.2% Tween (v/v). Blots were then incubated with primary antibodies for 1 hour at room temperature in PBS 0.02% Tween (anti-tubulin 1:10,000, anti-HSP-6 1:1000) or over-night at 4°C in 0.5% non-fat milk in PBS with 0.02% Tween (anti-GFP 1:1000, anti-HSP-16 1:1000) and then washed three times for 10 minutes with PBS-0.2% Tween. Membranes were then incubated for 1 hour at room temperature with HRP conjugated secondary antibodies in PBS 0.02% Tween, washed three times for 15 minutes in PBS-0.2% Tween, and then exposed to ECL Plus (Amersham) as per manufacturer's instructions to develop signal. Blots were imaged using a PXi multi-application gel imaging system (Syngene) and the densitometry of bands was quantified using image J. The anti HSP-6 antibody was generated by Thermo Scientific Open Biosystems by immunizing rabbits against a DAQEAKTAEEPKKEQN peptide corresponding to amino acids 642 - 657of the C-terminus of *C. elegans* HSP-6 (C37H5.8).

Genome-wide RNAi screening

Genome-wide screening was essentially conducted as previously described (Silva et al., 2011) using an RNAi library constructed by the Ahringer laboratory (Kamath et al., 2003) and consisting of bacteria expressing dsRNA against approximately 87% of predicted *C. elegans* genes. RNAi bacterial cultures were grown at 37°C with continuous shaking (315 rpm, Orbital shaker, GeneMachines HiGro, Genomic Solutions, USA) in 96 well plate format for 14 hours in 65 μl LB containing 50 μg/ml ampicillin. Bacterial cultures were then induced for 3 hours at 37°C with 0.5 mM of isopropyl β-D-thiogalatoside (IPTG, Sigma). Approximately 15 – 20 bleach synchronized wild type (N2)

L1 larvae were added to each well of a 96-well plate in 50 μ l of M9 containing 1 μ g/ml cholesterol, 50 μ g/ml ampicillin, 10 μ g/ml tetracycline, 0.1 μ g/ml fungizone and 170 μ g/ml IPTG and incubated at 20°C with continuous shaking (200 rpm, Innova 4430 Incubator Shaker, New Brunswick, USA). At day 2 of adulthood (70-74h post plating L1s) 96-well RNAi plates containing worms were placed in an incubator at 35°C in pre-warmed perspex boxes (approximately 6 plates per box) and heat shocked for 5 hours. Plates were then placed back at 20°C with continuous shaking and the population of each well was scored for motility (i.e. the proportion of worms exhibiting any form of thrashing regardless of speed) 24 hours later. RNAi clones were scored as hits in the preliminary round if \geq 30% of animals were moving. Preliminary hits (93 genes) were then re-arrayed on new 96-well plates and subjected to validation screening in liquid to remove false positives. Following this, final clones (51 genes) were screened three times on solid media and clones that restored movement to \geq 50% of the population in all 3 trials were taken as final hits.

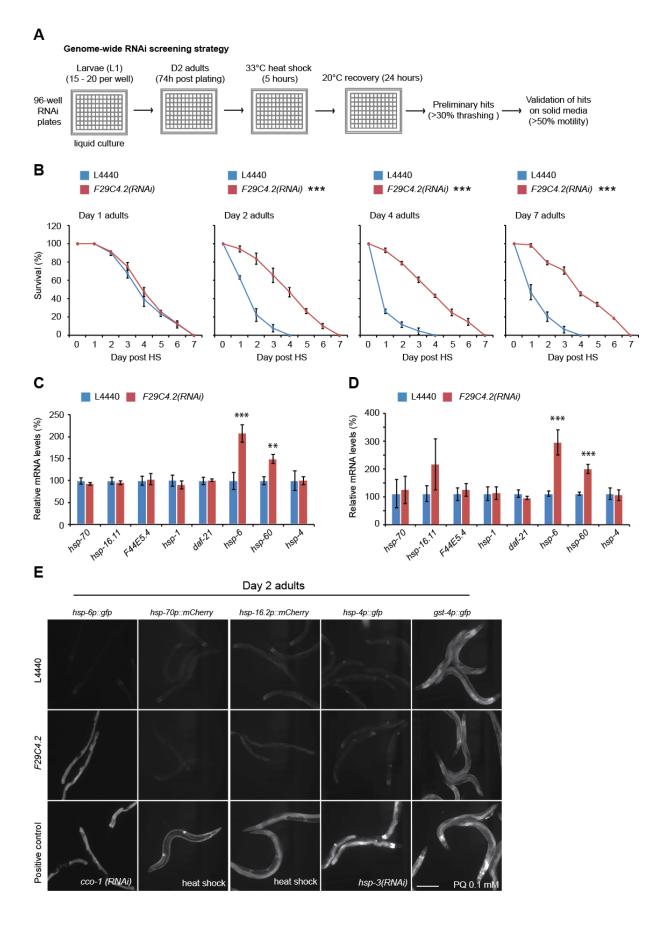
Oxygen Consumption and ATP measurements

Oxygen consumption was measured using a Seahorse XFe96 Analyzer at 25°C similar to that described previously (Andreux et al., 2014). In brief, worms raised on vector or RNAi bacteria were transferred onto empty plates at indicated stages and allowed to completely digest the remaining bacteria for 1 hour, after which 4 worms were transferred into each well of a 96-well microplate containing 200 µl M9 buffer. Basal respiration was measured for a total of 60 minutes, in 6 minute intervals that included a 2 minute mix, a 2 minute time delay and a 2 minute measurement. ATP levels were quantified using a luciferase based ATP detection kit (Thermo) as per manufacturer's instructions. Briefly, 10 µl of RIPA buffer lysate from 200 worms was incubated with luciferin and luciferase in ATP detection buffer without ATP. Luminescence was then measured using a Tecan M200 microplate reader and levels of ATP were calculated from a series of ATP standards of known concentration.

ROS measurements

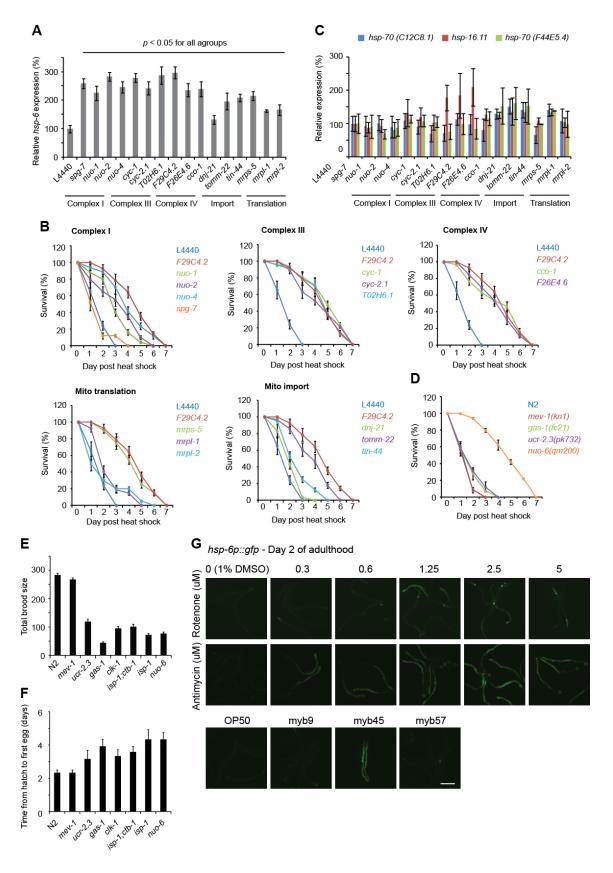
ROS levels were quantified in whole worms using the fluorescent substrate DHE (Thermo) (various forms of ROS), the Amplex-Red H₂O₂ detection kit (Thermo) (for hydrogen peroxide), or the Oxyblot detection system (Millipore) (for total carbonylated protein). DHE fluorescence was measured by placing 100 worms in M9 containing 4 µM DHE

(after washing twice in M9 to remove bacteria) and incubating in the dark with gentle agitation for 30 min at room temperature. Worms were then washed 3 times in M9 and placed in each well of a Costar 96-well, black, polystyrene plate (in 100 µl M9). The fluorescence intensity of each well was then measured (Ex/Em 358/461 non-oxidized; 518/606 oxidized) using a Tecan M200 plate reader. Background fluorescence (wells containing worms not treated with DHE) was subtracted from readings and the ratio of oxidized versus non-oxidized DHE was calculated for each treatment group. For H₂O₂ measurements, readings were collected as per manufacturer's instructions. Briefly, 50 µl of fresh lysate from 100 worms (in RIPA buffer) was added to 50 µl of Amplex-Red H₂O₂ detection reagent and incubated in the dark with gentle agitation for 30 min. Fluorescence intensity was then measured (Ex/Em 571/585) using a Tecan M200 plate reader. Background values were subtracted from readings and the amount of H₂O₂ present in each sample was calculated from a standard curve constructed from samples containing known quantities of H₂O₂. Levels of H₂O₂ were then normalized against total protein present in each sample, as determined by BCA assay (Pierce). Levels of carbonylated protein were measured using the Oxyblot system according to manufacturer's instructions. Briefly, 200 worms were lysed in RIPA buffer containing 50 mM DTT to prevent protein oxidation following lysis. 20 µl of each lysate was then incubated with 2,4-dinitrophenylhydrazine (DNPH) for 20 min at room temperature and separated by SDS-PAGE. Following western blotting, membranes were blocked with 5% milk-PBS and incubated for 1 hour at room temperature with rabbit anti-DNP primary antibody (1:150) in 0.02% PBS-Tween. Membranes were then washed three times with 0.2% PBS-Tween and incubated for 1 hour at room temperature with HRP conjugated goat anti-rabbit secondary antibody (1:300) in 0.02% PBS-Tween. Blots were then washed a further three times with 0.2% PBS-Tween and carbonylated proteins were detected using ECL reagent and an LAS4000 ImageQuant detection system (GE). Blots were stripped and re-probed for tubulin as a loading control (mouse antitubulin primary (Sigma, 1:5000)). Contrast and brightness were adjusted linearly across entire blots to enhance visibility of detected bands.



Supplemental figure 1, related to main figure 1. Knockdown of F29C4.2 constitutively activates the UPR^{mt} but not the UPR^{ER}, HSR or oxidative stress response

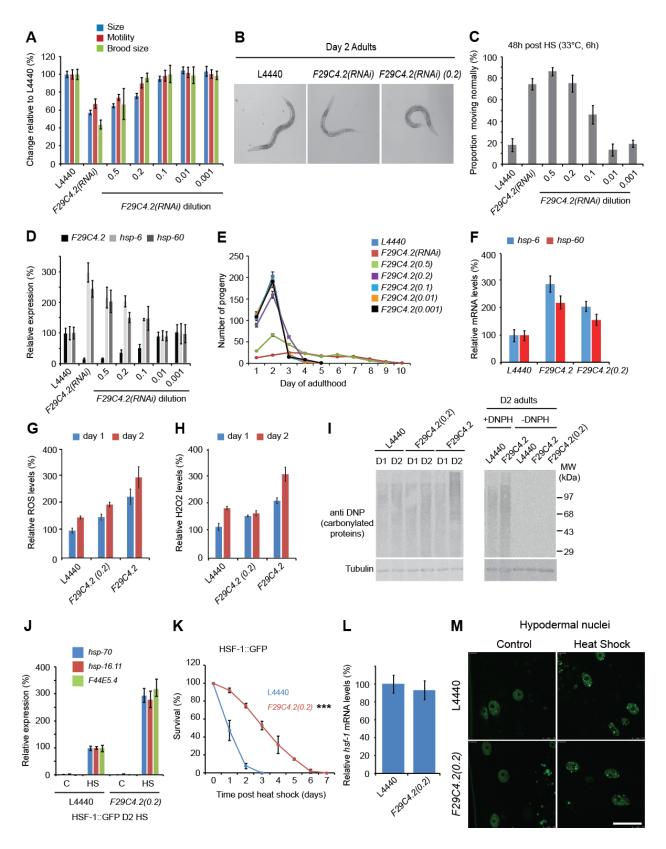
- (A) Schematic of genome-wide RNAi screening strategy to identify suppressors of stress resistance in reproductively active adults.
- **(B)** Survival of animals following a 4 hour, 35°C heat shock at day 1, 2, 4, or 7 of adulthood following growth on empty vector control (L4440) or F29C4.2(RNAi). Values are the mean of 4 biological replicates and error bars denote SEM. *** = p < 0.001. Statistical significance was calculated by two-way ANOVA with Bonferroni correction.
- (**C** and **D**) Basal expression of hsp-70(C12C8.1), hsp-16.11, F44E5.4, hsp-1, daf-21, hsp-6, hsp-60, and hsp-4 normalized to the housekeeping genes rpb-2 and cdc-42 at (C) day 1 or (D) day 2 of adulthood following growth on L4440 or F29C4.2(RNAi). Values are the mean of 4 biological replicates and error bars denote SEM. ** = p < 0.01, *** = p < 0.001. Statistical significance was calculated by ANOVA with Bonferroni correction.
- (E) GFP or mCherry fluorescence at day 2 of adulthood in UPR^{mt} (hsp-6p::gfp), UPR^{ER} (hsp-4p::gfp), oxidative stress response (gst-4p::gfp) and HSR (hsp-70p::mcherry and hsp-16.2p::mcherry) reporter lines grown on L4440 or F29C4.2(RNAi). Scale bar = 250 μ M.



Supplemental Figure 2, related to main figure 2. Mitochondrial perturbation activates the UPR^{mt} but not the HSR

- (A) Basal expression of *hsp-6* at day 2 of adulthood in animals grown on empty vector control (L4440) or RNAi against a mitochondrial AAA metalloprotease (*spg-7*), subunits of complex I (*nuo-1*, *nuo-2*, *and nuo-4*), complex III (*cyc-1*, *cyc-2.1*, *and T02H6.11*), and complex IV (*F29C4.2*, *F26E4.6*, *and cco-1*), or components of protein import (*dnj-21*, *tomm-22*, *and tin-44*), or mitochondrial translation (*mrps-5*, *mrpl-1*, *and mrpl-2*). Expression was calculated relative to the house keeping genes *rpb-2* and *cdc-42*. Values plotted are the mean of 4 biological replicates and bars represent SEM. *P* values were calculated by one-way ANOVA with tukey post analysis pairwise comparison of groups.
- (**B**) Survival following heat shock at 35°C for 4 hours on day 2 of adulthood in wild type (N2) animals grown on L4440 or RNAi against a mitochondrial AAA metalloprotease (*spg*-7), subunits of complex I (*nuo-1*, *nuo-2*, *and nuo-4*), complex III (*cyc-1*, *cyc-2.1*, *and T02H6.11*), and complex IV (*F29C4.2*, *F26E4.6*, *and cco-1*), protein import (*dnj-21*, *tomm-22*, *and tin-44*), or mitochondrial translation (*mrps-5*, *mrpl-1*, *and mrpl-2*). Values are plotted as mean survival at different days following HS. Error bars represent SEM. L4440 and *F29C4.2* curves are present on all graphs for reference.
- (C) hsp-70(C12C8.1), hsp-70(F44E5.4), and hsp-16.11 at day 2 of adulthood in animals grown on empty vector control (L4440) or RNAi against a mitochondrial AAA metalloprotease (spg-7), subunits of complex I (nuo-1, nuo-2, and nuo-4), complex III (cyc-1, cyc-2.1, and T02H6.11), and complex IV (F29C4.2, F26E4.6, and cco-1), or components of protein import (dnj-21, tomm-22, and tin-44), or mitochondrial translation (mrps-5, mrpl-1, and mrpl-2). Expression was calculated relative to the house keeping genes rpb-2 and cdc-42. Values plotted are the mean of 4 biological replicates and bars represent SEM.
- (**D**) Survival following heat shock at 35°C for 4 hours on day 2 of adulthood in wild type (N2), *mev-1(kn1)*, *ucr-2.3(pk732)*, *gas-1(fc21)*, and *nuo-6(qm200)* mitochondrial mutants. Values are the mean of 4 biological replicates plotted as mean survival at different days following HS. Error bars represent SEM.
- (E) Total brood sizes of wild type (N2) and mitochondrial mutant worms.

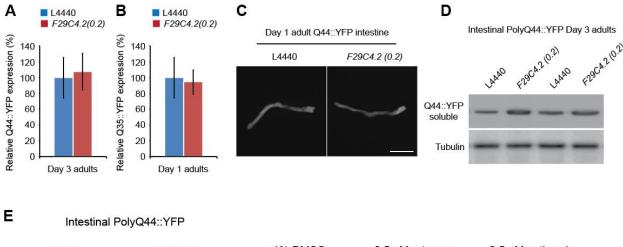
- **(F)** Number of days required post hatching for wild type or mitochondrial mutant worms to lay first eggs. In most cases this reflected delayed developmental rates, however, in the case of *ucr-2.3(pk732)* mutants, the delayed time to egg-lay reflected normal developmental timing to adulthood but a delayed appearance of embryos in the gonad.
- (G) GFP fluorescence at day 2 of adulthood in *hsp-6p::gfp* UPR^{mt} reporter worms following growth on plates containing 0, 0.3, 0.6, 1.25, 2.5, and 5 uM rotenone or antimycin, or plates seeded with OP50 (*E. coli*), MYb9 (*achromobacter*), MYb45 (*microbacterium*), or MYb57 (*stenotrophomonas*). Scale bar = 250 μM.

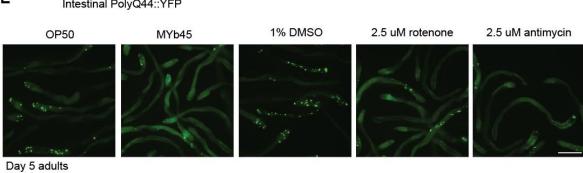


Supplemental figure 3, related to main figure 3. F29C4.2 knockdown influences animal physiology and stress resistance in a dose dependent manner

- (A and B) Relative (A) size, motility (body lengths per second), and total brood size with (B) representative brightfield images of worms on day 2 of adulthood. Brood size values are the mean of at least 20 animals. Error bars denote SEM.
- (C) Thermorecovery of N2 worms grown on L4440, F29C4.2(RNAi), or F29C4.2(RNAi) diluted 2, 5, 10, 100, and 1000 fold with L4440, 48 hours post heat shock at 33°C for 6 hours on day 2 of adulthood. Values are the mean of 4 biological replicates. Bars represent SEM.
- (**D**) *F29C4.2*, *hsp-6*, and *hsp-60* mRNA levels at day 2 of adulthood in animals grown on L4440 or *F29C4.2*(*RNAi*) diluted 2, 5, 10, 100, and 1000 fold with L4440. Values are the mean of 4 biological replicates.
- (E) Number of progeny produced during adulthood in animals grown on L4440, F29C4.2(RNAi), or F29C4.2(RNAi) diluted 2, 5, 10, 100, or 1000 fold. Values are the mean of 20 animals and bars represent SEM.
- (**F**) Relative *hsp-6* and *hsp-60* expression at day 2 of adulthood in L4440, *F29C4.2*, or *F29C4.2*(0.2) animals. Values were calculated relative to the housekeeping genes *rpb-2* and *cdc-42* and are the mean of 4 biological replicates. Bars represent SEM.
- (G) Levels of general ROS measured by dihydroxyethidium (DHE) oxidation on day 1 and day 2 of adulthood in animals grown on L4440, F29C4.2, or F29C4.2(0.2)(RNAi). Values are the mean of biological replicates and bars denote SEM.
- (H) Relative hydrogen peroxide (H_2O_2) levels at day 1 and day 2 of adulthood in L4440, F29C4.2, or F29C4.2(0.2)(RNAi) animals as measured by amplex-red fluorescence assay. Values are the mean of biological replicates and bars denote SEM.
- (I) Representative western blots of levels of carbonylated proteins at day 1 and day 2 of adulthood in L4440, F29C4.2, or F29C4.2(0.2)(RNAi) animals. Tubulin was probed as a loading control and DNPH samples demonstrate specificity of the anti-DNP antibody.
- (**J**) Relative expression of canonical HSR genes in HSF-1::GFP (AM1060) worms exposed to control or heat shock (33°C, 30 min) conditions at day 2 of adulthood. Values were calculated relative to the housekeeping genes *rpb-2* and *cdc-42* and are the mean of 4 biological replicates. Bars represent SEM.

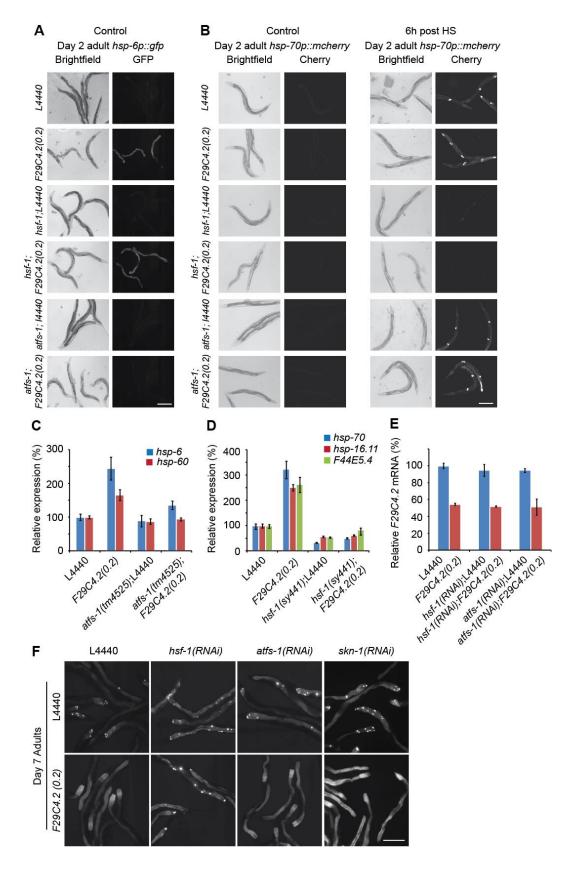
- (**K**) Survival of HSF-1::GFP worms at different days of recovery following 35°C heat shock for 4 hours on day 2 of adulthood. Values and are the mean of 4 biological replicates and bars represent SEM. Statistical significance was calculated by TWO-WAY ANOVA with Bonferroni correction. *** = p < 0.001.
- (L) Relative *hsf-1* mRNA levels on day 2 of adulthood in L4440, *F29C4.2*, or *F29C4.2*(0.2)(*RNAi*) treated animals. Values were calculated relative to the housekeeping genes *rpb-2* and *cdc-42* and are the mean of 4 biological replicates. Bars represent SEM.
- (M) Representative images of hypodermal nuclei of HSF-1::GFP worms grown on L4440 or *F29C4.2(0.2)(RNAi)* and exposed to control or HS conditions on day 2 of adulthood.





Supplemental figure 4, related to main figure 4. F29C4.2(0.2)(RNAi) does not alter expression of polyglutamine transgenes

- (A) Relative expression of polyQ(44)::YFP mRNA at day 3 of adulthood in L4440 and F29C4.2(0.2)(RNAi) treated animals. Values are the mean of 4 biological replicates and error bars denote SEM.
- **(B)** Relative expression of polyQ(35)::YFP mRNA at day 1 of adulthood in L4440 and F29C4.2(0.2)(RNAi) treated animals. Values are the mean of 4 biological replicates and error bars denote SEM.
- (C) Representative fluorescence images of day 1 adult worms expressing polyQ(44)::YFP in the intestine following growth on L4440 or F29C4.2(0.2)(RNAi). Scale bar = 250 μ M.
- (**D**) Western blots of soluble polyQ(44)::YFP and tubulin levels in day 3 adults following growth on L4440 or F29C4.2(0.2)(RNAi).
- (E) Representative images of vha-6p::polyQ(44)::YFP animals at day 5 of adulthood following exposure to vehicle (1% DMSO), 2.5 uM rotenone, 2.5 uM antimycin, OP50 or MYb45. Scale bar = 250 μ M.



Supplemental figure 5, related to main figure 5. Combinatorial RNAi with *atfs-1(RNAi)* or *hsf-1(RNAi)* suppresses the UPR^{mt} and HSR respectively but does not impair *F29C4.2* knock-down

- (A) Representative brightfield and fluorescence images of animals expressing *hsp-6p*::gfp under control conditions following growth on L4440, *F29C4.2(0.2)*, *hsf-1*;L4440, *hsf-1*;F29C4.2(0.2), *atfs-1*;L4440, or *atfs-1*;F29C4.2(0.2) RNAi. Scale Bar = 250 μM.
- (**B**) Representative brightfield and fluorescence images of animals expressing *hsp-70p*::mCherry under control conditions or 24 hours post heat shock (HS) following growth on L4440, *F29C4.2(0.2)*, *hsf-1*;L4440, *hsf-1;F29C4.2(0.2)*, *atfs-1;L4440*, or *atfs-1;F29C4.2(0.2)* RNAi. Scale Bar = 250 μM.
- (C) Relative expression of *hsp-6* and *hsp-60* on day 2 of adulthood in wild type or *atfs-1(tm4525)* mutants following growth on L4440 or *F29C4.2(0.2)(RNAi)*.
- (**D**) Relative expression of *hsp-70*, *hsp-16.11*, and *F44E5.4* in wild type or *hsf-1(sy441)* mutants following heat shock on day 2 of adulthood after growth on L4440 or *F29C4.2(0.2)(RNAi)*.
- (E) Relative expression of *F29C4.2* at day 2 of adulthood in animals grown on L4440, *F29C4.2*(0.2)(RNAi), *hsf-1*;L4440, *hsf-1*;F29C4.2(0.2), *atfs-1*;L4440, or *atfs-1*;F29C4.2(0.2) RNAi.
- (F) Representative images of the intestine of worms expressing Q44::YFP (vha-6::polyQ(44)::YFP) on day 7 of adulthood following growth on L4440, F29C4.2(0.2), hsf-1;L4440, hsf-1;F29C4.2(0.2), atfs-1;L4440, atfs-1;F29C4.2(0.2) RNAi, skn-1;L4440, or skn-1;F29C4.2(0.2) RNAi. Scale bar = 250 μ M.

Supplemental Table 1, related to main figure 1: Suppressors of thermorecovery in early adulthood

Gene	Human ortholog	Function
F29C4.2	COX6C	ETC complex IV
mtch-1	MTCH1/MTCH2	Mitochondrial carrier protein
C50F2.3	XAB2	Splicing factor
dct-19	EPHX1	Epoxide hydrolase
tba-8	TUBA8	Alpha tubulin
B0024.11	PUS7	Pseudouridine synthase
ubxn-6	UBXN6	Ubiquitin domain containing protein
slc-25A26	SLC25A26	S-adenosylmethionine transporter
twk-22	KCNK10	Potassium channel protein
dnj-7	DNAJC3	ER DNAJ chaperone
C53B4.4	PDZD8	Unknown

Supplemental Table 2, related to experimental procedures: Summary of primer pairs used in this study

Target	Forward	Reverse
hsp-70(C12C8.1)	CTACATGCAAAGCGATTGGA	GGCGTAGTCTTGTTCCCTTC
hsp-16.11	GGCTCAGATGGAACGTCAA	GCTTGAACTGCGAGACATTG
hsp-70(F44E5.4)	TGATACCCATCTCGGAGGAG	GTGGATTGGGTGAAATGTCC
rpb-2	AACTGGTATTGTGGATCAGGTG	TTTGACCGTGTCGAGATGC
cdc-42	GGTTGCTCCAGCTTCATTC	AACAAGAATGGGGTCTTTGA
hsp-4	GGGGACAATCATTGGTATCG	ACGCAACGTATGATGGAGTG
hsp-6	GTTATCGAGAACGCAGAAGGAG	CATCCTTAGTAGCTTGACGCTG
hsp-60	CATGCTCGTCGGAGTCAAC	TTTGTGATCTTTGGGCTTCC
gst-4	GCTACTTGGATAACCAGCTCCA	TCGTCTGGCATCAAAGAACAGT
F29C4.2	AAGACTGTTGCCGATTTGTAG	TTACGAACATTTTTATTCGGAACA
hsf-1	TGTACAAGGACGTCCCGAAT	TCCAAATTTTGTTGCGTCTG
daf-21	GACCAGAAACCCAGACGATATC	GAAGAGCACGGAATTCAAGTTG
hsp-1	ACTTCTACACCAACATCACTCG	CAAGGACGATGTCATGAACTTG
hsp-70 promoter	ATAGCATAGGCGACCCACAG	ACGTTCCTCTGGCATCTTCT
hsp-16.11 promoter	CTGAATGTGAGTCGCCCTCC	GAGAGCCTCTGCAAACTGGA
F44E5.4 promoter	CCAGCTGCATCACTCTGTCT	GGCCGACAGAAGAGACAACA
cdc-42 promoter	GTAAAGAAACGCTCGTGGCA	GATCGTCTGCATTTCGCCTG